
Effects of Different Packaging Materials Coated with *aloe vera* Extract on the Microbial Quality of African Breadfruit Flour (*treculia africana*) during Storage

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ABSTRACT

Antimicrobials in food packaging are used to enhance quality and safety by reducing surface contamination of processed food. This study investigated the effects of *Aloe vera*- coated packaging materials on the microbial quality characteristics of breadfruit flour. Breadfruit flour was packaged in Jute bag (JB), Kalico bag (KA), low-density Polyethylene-lined Brown paper (LDPEBP) and they were compared with control. Samples were analyzed for changes in microbiological (total colony count and total fungal count) and moisture content during a storage period of 12 weeks interval during storage at ambient temperature of $25 \pm 2^\circ\text{C}$. Packaging significantly ($p < 0.05$) affected the moisture content and microbiological of breadfruit flour during storage. The moisture content, total colony count, and total fungi count significantly ($p < 0.05$) increased as the storage time increased. The sample packaged in Brown paper (BP) were more acceptable than those in other packaging materials.

Keywords: Breadfruit Flour; Packaging Materials; Storage PEriod; *Aloe Vera* Extract; Food Safety; Microbial Load

1. Introduction

Active Packaging (AP) is a modern development consisting of a group of techniques in which the package is self-motivated and is actively involved with food products or act together with internal atmosphere to extend the shelf life while maintaining quality and safety. Active packaging is sometimes referred to as interactive or smart packaging which is planned to sense internal or external environmental changes and to take action by changing its own properties or attributes. Potential techniques used in active packaging are the use of oxygen scavenging/carbon dioxide, ethylene and moisture absorbing systems by placing sachets, incorporation of antimicrobial agents onto polymer surfaces or in plastics films, sheets or on materials and into the pads for fresh produce (John, 2008).

Packaging is a means of providing the correct environmental conditions for food during storage and the choice of materials for packaging depends on the nature of the product, the storage and handling conditions (temperature, humidity, risk of physical deterioration) among other factors (Brown, 1992). Adetunji *et al.* (2012) found that it was better to store gari in polythene lined brown multiply paper than in polythene lined kalico. Age long chemicals have been used to control pathogens, however, in the recent time, pathogens have developed resistance. Therefore, there is a need for alternative approach to plant diseases control such as the use of plant extracts. Plant extracts are eco-friendly, accessible to rural dwellers, cost effective and more or less phytotoxic. Plant extracts have been successfully used to control a number of plant diseases (Okigbo, 2009).

Antimicrobials in food packaging are used to enhance quality and safety by reducing surface contamination of processed food; they are not a substitute for good sanitation practices (Brody et al., 2001; Cooksey, 2005). Antimicrobials reduce the growth rate and maximum population of microorganisms (spoilage and pathogenic) by extending the lag phase of microbes or inactivating them (Quintavalla and Vicini, 2002). Antimicrobial agents may be incorporated directly into packaging materials for slow release to the food surface or may be used in vapour form. African breadfruit (*Treculia africana*) is a tropical tree crop belonging to the taxonomic family moraceae, genus, *Treculia* (Enibe et al., 2003). The family consists of about 50 genera and over 1000 species. It is high yielding with an average sized tree producing 400-600 fruits per year. Yields are superior to other starchy staples due in part to their verticality of production (NTBG, 2009). Singh (2009) reported that a single tree produces between 150 and 200 kg of food whereas Morton (1987) reported yields between 16 and 32 ton/ha/year.

Notwithstanding the high yielding potential, the crop is underutilized and considered as less important. The high water activity of the crop makes it easily susceptible to microbial attack (Amusa et al., 2002) as well as the bulky nature makes transportation difficult. This has prompted processing of the fruit into products such as flour. The production of breadfruit flour has shown to be useful technique in extending the shelf life. Conventional flours are known to play important functional roles in food systems. However, their rising cost has resulted in the search for alternative replacements to fully or partially substitute the conventional flours with non-conventional in foods has been reported (Chillo et al., 2008).

Therefore, this work was designed to evaluate the effects of *Aloe vera*-coated packaging materials on the microbial quality characteristics of breadfruit flour during storage.

2. Materials and methods

2.1 Sample Collection and Preparation

Treculia africana fruits were collected from a local farm in Ilesha, Osun State, Nigeria. Fresh, firm and mature *Treculia africana* fruits were harvested and washed under running clean water and transported to laboratory for analysis. Fresh leaf samples of *Aloe vera* was obtained from the experimental farm of Nigerian Stored Products Research Institutes, Ilorin, Kwara State, Nigeria. The plants was identified at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Nigeria. The plants were dried in the sun until the moisture content was reduced. The plant was then pounded in a mortar, and further ground to powder using a clean electric blender and stored in polythene bags until use.

2.2 Cold Aqueous Extracts of *Aloe vera*

Fifty grams of the finely ground powder was introduced into a conical flask and 200 ml of absolute ethanol was added to the ground *Aloe vera* respectively. The mixture was put in a conical flask and placed on a mechanical shaker. After 48 hrs, the extract was decanted and passed through a muslin cloth and later filtered with a Whatman No.1 filter paper (110 mm). The filtrate obtained was evaporated to dryness at 45 °C, and the residue obtained was reconstituted with aqueous water as stock concentration of 250 mg/ml.

2.3 Preparation of *Treculia africana* flour

The freshly harvested *Treculia africana* fruits were peeled and sliced into cubes (2 cm³) under running tap water. The sliced pieces were dried in an oven at 50 °C for 24 hours and then cooled to room temperature (28 °C). A hammer mill was used to mill the dried chips. The resultant flour was sieved through 75 µm mesh and packaged in a sealed plastic bottle prior to analysis.

2.4 Storage of *Treculia africana* flour in different packaging materials

A 25 kg sample of *Treculia africana* flour was stored in Jute bag (JB), Kalico bag (KA), low-density Polyethylene in Brown paper (LDPEBP) while the control contained 25 kg of *Treculia africana* flour in an unsealed jute bags respectively. They were then stored at ambient temperature (30 ± 3 °C) for the period of 13 weeks. Thermohydrograph was placed in the storage room to record the temperature and relative humidity of the atmosphere.

2.5 Application of *Aloe vera* extract to the packaging materials

The different packaging materials Jute bag (JB), Kalico bag (KA), low-density polyethylene brown paper

(LDPEBP) were coated with 250 mg/ml of *Aloe vera* extract. They were then air dried at ambient temperature (30 ± 3 °C), while the uncoated jute bag served as the control.

2.6 Preparation of media

The materials used such as glass wares were properly sterilized in the oven (Gallenkamp) at 160 °C for 1 h. All the media used were prepared according to the manufacturer's instructions and then autoclaved at 121°C for 15 min.

2.7 Isolation and characterization of pure cultures of microorganisms

One gram of sample was weighed and crushed to powder with sterile mortar and pestle. It was then placed in a sterile test tube and dissolved with 10 ml of distilled water to make the stock. The suspension was filtered through sterile glass wool. Serial dilution was done to the necessary dilution factors and pour-plated. The plates were left to gel and then incubated. The bacteria plates were incubated at 37 °C for 48 h on nutrient agar while the fungal plates were incubated at 25 °C for 72 h on potatoes dextrose agar. At the end of each incubation period, the colonies were counted and sub-cultured onto fresh media maintained on slants from nutrient agar and preserved at 4 °C in the refrigerator according to Fawole and Oso (2004). Tentative identification of bacterial isolates was done using the Bergey's Manual of Determinative Bacteriology (Bucchanans and Gibbons, 1974). Fungal identification was carried out according to the procedure described by (Samson and Van Reenen-Hoekstra, 1982).

2.8 Moisture content

The moisture content of the samples was determined by the standard method of AOAC (2000). Five grams each of all the samples was weighed into the preset oven and the drying was performed at 105 °C for 4 hrs to constant weight. They were removed from the desiccators to cool and then weighed. The difference in weight was used to obtain the moisture content. All analysis were carried out in duplicate. The percentage moisture content was then calculated as Moisture content MC % = Weight loss / Original weight X 100 %

2.9 Data analysis

Data collected on each treatment were analyzed using Analysis of Variance (ANOVA), while means with significant differences were separated using Duncan Multiple Range Test (DMRT). All analyses were carried out using SPSS version 16 software package.

3. Result and Discusion

During this study the following bacteria were isolated before storage in the different packaging materials: *Proteus vulgaris*, *Lactobacillus spp*, *Bacillus substilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium spp* and *Bacillus cereus* while the fungus isolated were *Aspergillus niger*, *Fusarium oxysporium*, *Rhizopus stoloniper*, *Aspergillus fumigatus* and *Saccharomyces cerevisae*. (Tables 1 and 2). The result of the total colony count in figure 1 showed that the breadfruit flour stored in low-density Polyethylene in Brown paper (LDPEBP) had the lowest number of colony forming units of 3.17×10^4 CFUg⁻¹ followed by Kalico bag (KA), Jute bag (JB) which had colony forming unit of 4.15×10^4 CFUg⁻¹ and 5.93×10^4 CFUg⁻¹ respectively compared to the control that had the highest, colony forming unit of 9.97×10^4 CFUg⁻¹ at the end of storage at ambient condition. The results from figure 1, clearly indicated a decrease in the number of colony forming units of bacteria in the low-density Polyethylene in Brown paper (LDPEBP) < Kalico bag (KA) < Jute bag (JB) when compared to the control sample after 13 weeks of storage. This could be attributed to the availability of moisture suitable for microbial activities because there was as much as (two times the initial moisture content) in those samples by 13 weeks of storage. According to Jay (1992), bacteria require relatively high levels of moisture for their growth. The higher numbers estimated could also be due to the inability of those packages to serve as a physical barrier to oxygen, which is essential for carrying out metabolic activities by micro-organisms. The lower bacterial numbers recorded in low-density Polyethylene in Brown paper (LDPEBP) packages could be attributed to its good moisture barrier properties and also because the packaging material could have acted as effective physical barrier against bacteria. A variety of microbes find their way into foods, introduced from the soil in which they were grown, and during harvest, packaging, storage and handling (ICMSF, 1996).

The result of the total fungal count in figure 2 showed that the breadfruit flour stored in low-density Polyethylene in Brown paper (LDPEBP) had the lowest number of colony forming units of 3.17×10^6 CFUg⁻¹ followed by Kalico bag (KA), Jute bag (JB) which had colony forming unit of 3.63×10^6 CFUg⁻¹ and 5.67×10^6 CFUg⁻¹ respectively compared to the control that had the highest, colony forming unit of 9.78×10^6 CFUg⁻¹ at the end of storage at ambient condition. Most fungal growth occurs at a water activity (aw) as low as 0.80, which explains why dried foods often become mouldy (Nesta *et al.*, 2001). If the water activity of a dehydrated product is allowed to rise above a certain critical level, microbiological spoilage may occur. In such cases a packaging material with a low permeability to water vapour and effectively sealed, is required (Brennan, 2006). Hosoney (1994) worked on wheat flour and reported that at lower moisture, fungi did not grow but at about 14 % moisture content or slightly above, fungal growth took place. The increased in gaseous exchange between the stored product and the environment around the packaging material from Kalico bag (KA), Jute bag (JB) and control also lead to increase in moisture content value, total fungal count of breadfruit flour. It has been reported that fungal growth in agricultural produce is directly correlated to the moisture content (Adeniji, 1996). The typical water activity which is necessary for fungal growth ranges from 0.70-0.90 (Frazier and Westhoff, 2003).

Appropriate coatings can sometimes impart antimicrobial effectiveness. An *et al.* (2000) claimed that a polymer-based solution coating would be the most desirable method in terms of stability and adhesiveness of attaching a bacteriocin to a plastic film. It was found that low-density polyethylene (LDPE) films coated with a mixture of polyamide resin in i-propanol/n-propanol and a bacteriocin solution provided antimicrobial activity against *Micrococcus flavus*. The migration of bacteriocins reached equilibrium within 3 d, but the level attained was too low to affect several bacterial strains spread on an agar plate media. When the films were in contact with a phosphate buffer solution containing strains of *M. flavus* and *L. monocytogenes*, a marked inhibition of microbial growth of both strains was observed. LDPE film was successfully coated with nisin using methylcellulose (MC)/ hydroxypropyl methylcellulose (HPMC) as a carrier. Nisin was found to be effective in suppressing *S. aureus* and *L. monocytogenes* respectively (Cooksey, 2000).

According to Hong *et al.* (2000), the antimicrobial activities activity of 5.0 % w/w Propolis extract, Chitosan polymer and oligomer, or Clove extract in LDPE films (0.030- to 0.040-mm thick) against *Lactobacillus plantarum*, *E. coli*, *S. cerevisiae*, and *Fusarium oxysporum* is best determined through viable cell counts. Overall, LDPE films with incorporated natural compounds show a positive antimicrobial effect against *L. plantarum* and *F. oxysporum*. Preliminarily studies by Suppakul *et al.* (2002) with LLDPE films (45 to 50 µm thick) containing 0.05 % w/w linalool or methyl chavicol showed a positive activity against *E. coli*.

The direct incorporation of antimicrobial additives in packaging films is a convenient means by which antimicrobial activity can be achieved. Several compounds have been proposed and/or tested for antimicrobial packaging using this method. Han and Flores (1997) studied the incorporation of 1.0 % w/w potassium sorbate in LDPE films. A 0.1 mm thick film was used for physical measurements, while a 0.4 mm thick film was used for antimicrobial effectiveness tests. It was found that potassium sorbate lowered the growth rate and maximum growth of yeast, and lengthened the lag period before mold growth became apparent.

Paik *et al.* (1998) and Shearer *et al.* (2000) observed a decrease in all bacterial cells, including *S. aureus*, *Pseudomonas fluorescens*, and *E. faecalis* in bulk fluid when using an antimicrobial nylon film. The results indicate that this decrease is more probably to be due to the bactericidal action than to surface adsorption (Paik *et al.*, 1998). Although the mechanism of the reduction in the bacteria population remained uncertain, electrostatic attractive forces between the positively charged film surface and the negatively charged *E. coli* and *S. aureus* were presumed to be the reason for this effect (Shearer *et al.*, 2000).

| Isolate | Cellular shape | Colonial elevation | Colonial edge | Colonial opacity | Colonial surface | Colonial pigmentation | Cellular arrangement | Gram's staining | Motility test | Spore staining | Capsule staining | Catalase test | Methyl red test | Starch hydrolysis | Citrate utilization | Oxygen reaction | Action on simple carbohydrates | | | | | probable microorganism |
|---------|----------------|--------------------|---------------|------------------|------------------|-----------------------|----------------------|-----------------|---------------|----------------|------------------|---------------|-----------------|-------------------|---------------------|-----------------|--------------------------------|---------|---------|---------|----------|------------------------|
| | | | | | | | | | | | | | | | | | Lactose | Glucose | Sucrose | Maltose | Fructose | |
| 1 | Rod | Raised | Entire | Translucent | Smooth | Cream | Chain | -ve | -ve | -ve | + | -ve | -ve | + | + | F/A/N | -ve | -ve | A/G | A/G | A/G | Proteus vulgaris |
| 2 | Rod | Raised | Lobate | Opaque | Smooth | Creamy White | Clusters | + | -ve | -ve | -ve | -ve | -ve | -ve | -ve | F/A/N | A | A | A | A | A/G | Lactobacillus species |
| 3 | Rod | Flat | Lobate | Opaque | Dull | White | Clusters | + | + | + | + | + | + | -ve | -ve | F/A/N | A/G | A | A | A | A | Bacillus subtilis |
| 4 | Cocci | Raised | Entire | Opaque | Smooth | Creamy White | Clusters | + | -ve | -ve | -ve | + | -ve | + | + | F/A/N | A/G | A | A | A | -ve | Staphylococcus aureus |
| 5 | Rod | Raised | Entire | Translucent | Smooth | Yellowish Cream | Chain | -ve | + | -ve | -ve | -ve | + | -ve | -ve | A/E | -ve | A | A | A | A/G | Pseudomonas aeruginosa |
| 6 | Rod | Raised | Entire | Opaque | Rough | Cream | Chain | -ve | -ve | + | -ve | -ve | -ve | + | -ve | A/N | A | A/G | A | A/G | A | Clostridium species |
| 7 | Rod | Raised | Lobate | translucent | Dull | Cream | chain | + | + | + | + | + | + | + | -ve | A/E | -ve | A/G | A/G | A/G | A/G | Bacillus cereus |

| Isolate | Description | fungi |
|---------|--|--------------------------------|
| 1 | Colonies at 25 °C attaining a diameter of 4-5cm within 7 days, usually consisting of a compact white or yellow basal felt with a dense layer of dark brown to black conidiophres. Conidial heads, radiate, tending to split into loose columns with age. Conidophore stipes smooth-walled, hyaline but often in brown colours. Vesicles globose to subglobose. | <i>Aspergillus niger</i> |
| 2 | Colony at 25°C attaining a diam. of 4.5 cm in 4 days. Aerial mycelium sparse or floccose, becoming felting, whitish or peach, usually with the purple tinge more intensified near the medium surface. Variable in shape and size, ovoid-ellipsoidal to cylindrical, straight or slightly curved. Conidiophores are usually short branched on phialides | <i>Fusarium oxysporium</i> |
| 3 | Colony whitish becoming grayish-bromnish sporangiospores and brown-black sporangia, often over 20mm high. Sporangiospores is a colourless to dark brown, smooth or slightly rough-walled stolons opposite the branched rhizoids. Sporangia globose to subglobose, ovoid, blackish-brown at maturity. | <i>Rhizopus stoloniper</i> |
| 4 | Colonies on 25 °C attaining a diam. of 3-5cm within 7 days, usually of a dense felt of yellow-green conidiophores. Conidia heads typically radiates latter splitting in several loose columns, yellow-green becoming dark yellow-green. Sclerotia often produced in fresh isolates, variable in shape and dimension often brown to black. | <i>Aspergillus fumigatus</i> |
| 5 | Colonies of Saccharomyces grow rapidly and mature in three days. They are flat, smooth, moist, glistening or dull, and cream to tannish cream in color. Blastconidia (cell buds) are observed. They are unicellular, globose, and ellipsoid to elongate in | <i>Saccharomyces cerevisae</i> |

6 shape. Multilateral (multipolar) budding is typical. Colonies at 25 °C attaining a diameter of 3-5cm within 7 days, usually consisting of a dense felt of yellow-green conidiophres. Conidia heads, radiate tending to split into loose columns with age. Conidiophores stipes smooth-walled, hyaline but often in brown colours. *Aspergillus flavus*

Table 2. Fungus isolated from *Treculia Africana*

Key:

-ve = Negative AE = Aerobic AN = Anaerobic A = Acid production
 +ve = Positive FAN = Facultative anaerobe AG = Acid and Gas production

Table 1. Colonial morphology, cellular morphology and biochemical characteristics of the bacterial isolates from *Treculia africana* flour.

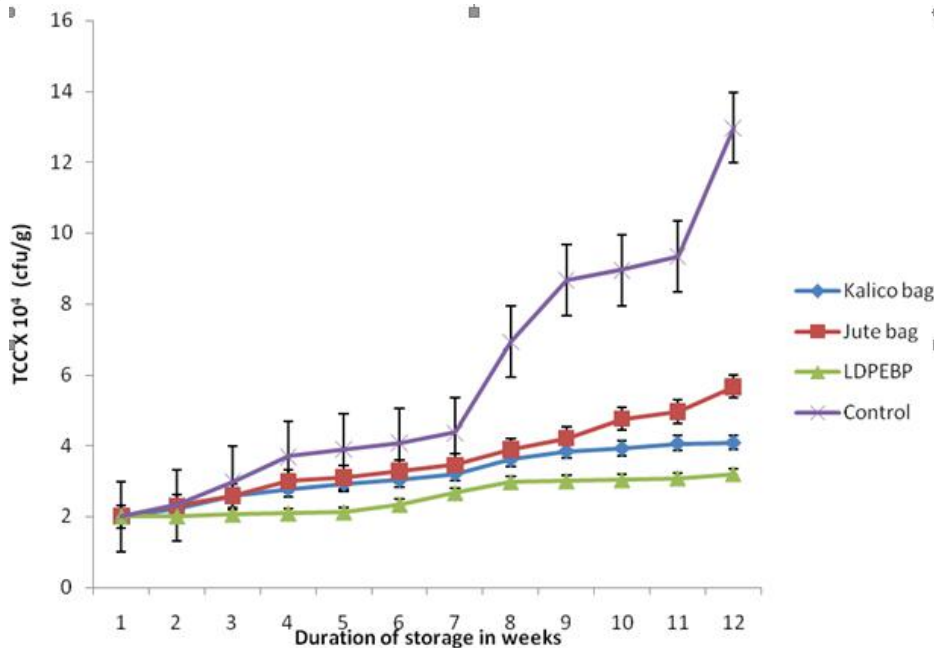


Figure 1; Total colony count of breadfruit flour.

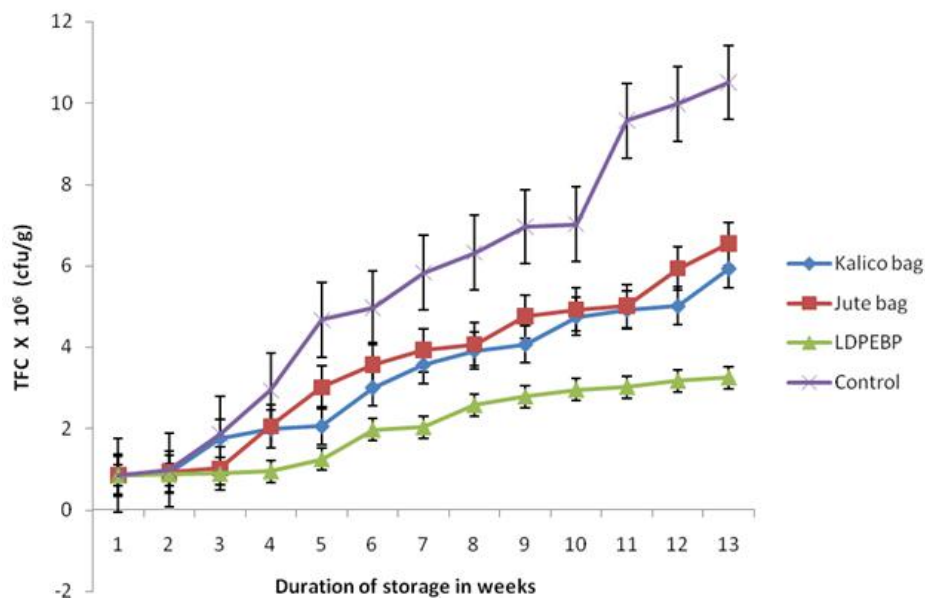


Figure 2; Total fungi count in breadfruit flour.

The result of the percentage moisture content in figure 3 showed that the breadfruit flour stored in low-density Polyethylene in Brown paper (LDPEBP) had the lowest moisture content of 7.9% followed by Kalico bag (KA), Jute bag (JB) which had 10.80 % and 13.00 % respectively compared to the control that had the highest moisture content of 15.70 % at the end of storage at ambient condition. All the breadfruit flour stored in Kalico bag (KA), Jute bag (JB) had high microbial counts compared to Low-density Polyethylene in Brown paper (LDPEBP) while their

moisture content were within the range of recommended value for flour samples of 7-13 % (Christensen and Kaumann, 1973). There is a linear relationship between the incidence of different types of microorganisms and moisture content of samples, as breadfruit flour is a rich carbohydrate source for yeast and mould growth (Uraih and Ogbadu, 1980). Moisture content of hygroscopic material such as dry food is in direct relation to the humidity of the surrounding air (Wilhelm *et al.*, 2004). Generally, changes in moisture content in all the samples during the storage period were due to changes in humidity of the storage atmosphere. Low-density Polyethylene in Brown paper (LDPEBP) packages had the least moisture values because they had better moisture barriers when compared to Kalico bag (KA), Jute bag (JB) packages which allowed moisture in and out of them. The higher moisture content in control packages could be attributed to their poor moisture resistance ability.

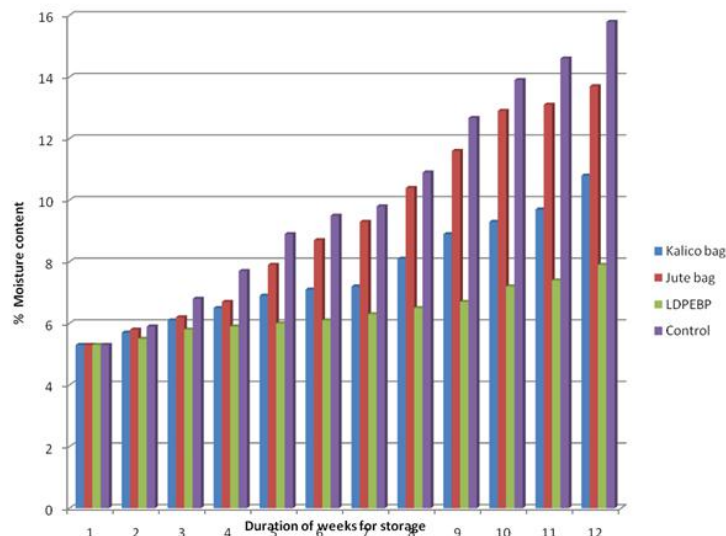


Figure 3; Moisture content percent of breadfruit flour in different packaging materials.

4. Conclusion

This study has revealed the potential of botanicals in the extension of the shelf life of African Breadfruit Flour. This will go a long way in providing better alternative to over dependency on synthetic fungicides used on some packaging materials. The use of plant products, such as *Aloe vera* in spoilage microorganisms control could reduce over reliance on chemicals, as well as cut down production cost. This will also go a long way in the minimizing the effect of pesticide residue experienced when chemical pesticides are applied to packaging materials. Moreover, the simple extraction method of the *Aloe vera* extract and its readily availability will enable its easy adaptability and will go along way in reduction of food losses and wastage.

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Conflict of Interest

We declare that we have no conflict of interest

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